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(54) Title: FACTOR VII-DERIVED PEPTIDES

(57) Abstract

The present invention relates to compounds comprising the amino acid sequences of the formulae (IA): -CVNENGGCEQYCSD-, (IB): -FCLPAFEGRNCE- and/or (IC): -RCHEGYSLLADGVSCT- as well as peptide fragments thereof, esters, amides, salts and cyclic derivatives thereof, functional analogues thereof and extended peptide chains carrying amino acids or peptides at the termini of the above sequences or fragments, for use in the prevention or inhibition of binding of tissue factor to FVII.

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FACTOR VII-DERIVED PEPTIDES.

The present invention is concerned with peptide
reagents and compositions thereof which reduce blood
5   clot formation.

Blood clotting relies upon a series or cascade of
activating reactions to produce the ultimate fibrin
clot. The cascade leading to fibrin formation may be
triggered initially in two different ways - by contact
10   with abnormal surfaces (the "intrinsic pathway") or by
traumatization of blood vessels which causes secretion
of the lipoprotein known as "tissue factor" or TF (the
"extrinsic pathway"). The present invention is
primarily concerned with the extrinsic blood clotting
15   pathway.

TF is an integral membrane protein which appears on
many cell types. However, cells which constitutively
express TF, for example the muscle cells of vessels
intima, are not normally exposed to blood (see Edgington
20   et al., Thromb. Haemostas. 66(1): 67-69 (1991)). Thus
initiation of the extrinsic blood clotting pathway
appears to require either the disruption of blood vessel
walls (see Almus et al., Blood 76: 354-360 (1990))
and/or activation of endothelial cells or monocytes to
25   express TF (see Edwards et al., Blood 54: 359-370 (1979)
and Bevilaqua et al., PNAS USA 83: 4533-4537 (1986)).
Disruption of the blood vessel wall may occur due to
fissuring of an atherosclerotic plaque which exposes
tissue macrophages and smooth muscle cells to the blood
30   (see Wilcox et al., PNAS USA 86: 2839-2843 (1989)). TF
may also be exposed following injury to blood vessels
during thrombolytic therapy, surgery for grafting,
mechanical restoration of vessel patency or other
similar techniques. On the other hand, TF expression in
35   endothelial cells or in monocytes may be induced during
sepsis due to production of tumour necrosis factor-α or

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interleukin-1 (see Edwards et al., supra and Gregory et al., J. Clin. Invest. 76: 2440-2445 (1985)).

The serine protease Factor VIIa (FVIIa) is involved in the extrinsic blood clotting pathway. FVIIa is formed by proteolysis from its inactive pro-enzyme Factor VII (FVII) by other participants in the blood clotting process, including Factor Xa, Factor XIIa, Factor IXa or thrombin. Activation of FVII to FVIIa has been reported to be markedly enhanced when FVII is bound to its co-factor tissue factor (TF) (see Nemerson, Semin. Hematol. 29(3): 170-176 (1992)). Yamamoto et al. have also suggested that conversion of FVII to FVIIa may be autocatalytic (see J. Biol. Chem. 267(27): 19089-19094 (1992)).

FVIIa forms a complex with TF in the presence of calcium ions and the FVIIa/TF complex catalyses the conversion of Factor X to its active form, Factor Xa, in the next step of the blood clotting process via the extrinsic pathway.

The structure of FVII has been investigated and the cDNA sequence was reported by Hagen et al. in PNAS USA 83: 2412-2416 (1986). FVII is a vitamin K dependent protein and, by analogy to other vitamin K dependent proteins, a putative γ-carboxyglutamic acid (Gla) domain has been identified at the amino terminal. It was predicted, again by analogy to the other vitamin K proteins, that the Gla domain was required for binding to TF (see Hagen et al., supra). The Gla domain is followed by two potential growth factor (GF) domains. However, the literature has not suggested any function for the GF domains.

Activation of the extrinsic pathway for blood clot formation has been suggested as the primary event leading to fibrin formation (see Weiss et al., Blood 71: 629-635 (1988) and Weiss et al., Blood 73: 968-975 (1989)) and is thus of prime importance in the pathogenesis of arteriosclerotic lesions and in

reocculusion and and restenosis following
endarterectomy. However effective therapeutic agents
able to intervene in the activation of this pathway are
not available, despite demand (see Shepard, TIBTECH 9:
80-85 (1991)).

The present invention provides peptides and
analogues or salts thereof which inhibit the association
of FVII or FVIIa with TF. Through the action of the
peptides according to the invention, formation of the
FVIIa/TF complex is limited and therefore activation of
Factor X is reduced.

Certain peptides stated to be useful in blood
clotting therapy are disclosed in WO-A-91/07432 of the
Board of Regents, The University of Texas System. The
peptides disclosed either occur in the region between
the Gla and the first GF domains or in the catalytic
domain of FVII or FVIIa. Although inhibition of
FVIIa/TF complex formation is discussed, those peptides
disclosed in WO-A-91/07432 which cause such an effect do
so via inhibition of the Gla function. Such peptides
are thus unspecific in their action since other
physiological proteins have Gla domains, for example
protein C which has close sequence homology to the Gla
domain of FVII. Hence, the function of protein C would
also be disturbed by peptides disclosed in WO-A-91/07432
in an undesirable way.

In WO-A-90/03390, Corvas Inc. suggest that certain
peptides derived from the amino acid sequence of FVII
(or FVIIa) might be useful in preventing the action of
the fully formed FVIIa/TF complex. Two particular
peptide sequences were disclosed in WO-A-90/03390 as
being active in this respect. The sequence -VGHFGV- is
based upon amino acids nos. 372-377 of FVII which are
situated near the carboxy terminus. The other sequence
is -SDHTGTKRSCR- which is located at amino acids nos.
103-113 of FVII and is part of the second GF domain.
Corvas Inc indicate that these peptides, and analogues

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thereof, inhibit the cascade reaction initiated by the
FVIIa/TF complex.

Furthermore, in Table 1, on page 14 of WO90/03390,
it is indicated that of the various regions of the
second GF domain, only SDHTGTKRSC (103 to 112) was
active and that the other regions, namely from amino
acids 50 to 101 and 114 to 127, were totally inactive in
inhibition of activation of Factor X by Factor VII and
tissue factor.

However, we have shown that, contrary to the
reported findings of Corvas Inc., the region SDHTGTKRSC
from 103 to 112 is a rather poor inhibitor of the
binding of Factor VII to tissue factor and that certain
of the regions said to be inactive are, in fact, highly
active.

Our initial finding was that a point mutation in
FVII, wherein the amino acid glutamine at position 100
was replaced by arginine, was observed in 16 FVII
deficient Norwegian patients and suggested that this
region of the FVII molecule is important for FVII
activity. We established firstly that the amino acid
sequence 91-102 was particularly active and that this
could usefully also include amino acids 103 and 104. We
then found that the amino acid sequence 114-127, also in
the GF domain, was relatively active while the Corvas
sequence 103-112 had only moderate activity.

Subsequently, we found that the amino acid sequence
72-81 acted synergistically in enhancing the inhibitory
action of the peptides of the sequence 91 to 104, and
primary utility of peptides derived from the region 72-
81 is in such synergistic combinations.

Furthermore, fragments of the above sequences have
been found to be inhibitory and in general fragments of
5 or more amino acids from the above sequences will be
useful in the inhibition of FVII activity.

The present invention thus concerns peptides
comprising the amino acid sequences of formulae

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-CVNENGGCEQYCSD-	IA
-FCLPAFEGRNCE- and/or	IB
-RCHEGYSLLADGVSCT-	IC

as well as peptide fragments thereof, especially
fragments having 5 or more amino acids, esters, amides
salts and cyclic derivatives thereof, functional
analogues thereof and extended peptide chains carrying
amino acids or peptides at the termini of the above
sequences or fragments.

According to one feature of the invention we
provide peptides of the sequences IA, IB and/or IC as
defined above, with the exclusion of the peptides

CVNENGGCEQYC	IIA
CLPAFEGRNC and	IIB
CHEGYSLLADGVSC	IIC

Where appropriate, compounds of formula IA, IB and IC
and their various derivatives and fragments are termed,
for convenience, peptide IA compounds, peptide IB
compounds and peptide IC compounds.

Fragments of the peptide sequences of formulae IA,
IB and/or IC include, in particular

-VNENG-	-NGGCEQYCSD-
-ENGGC-	-GGCEQYCSD-
-GGCEQ-	
-CEQYV-	

Our studies have shown that amino acid 95 is
important in the peptides of the amino acid sequence 91
to 104 and the peptide of sequence 95 to 104 is the most
active inhibitor so far tested. This is NGGCEQYCSD.

The peptides of formulae IIA, IIB and IIC are
excluded on the basis that they were disclosed in
WO90/03390 of Corvas Inc., although stated to have zero

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inhibitory acticity. There is clearly no suggestion in
WO90/03390 that there would be any benefit in making any
of the related peptides, including fragments or
extensions thereof, for use in combatting blood clotting
disorders.

On the basis that WO90/03390 incorrectly reported
zero inhibitory activity for the peptides of formulae
IIA and IIB one aspect of the present invention provides
the therapeutic or diagnostic use of the compounds of
formulae IA and/or IB as defined above to bind to tissue
factor and thus prevent or inhibit binding of tissue
factor to FVII. In the case of peptide IC compounds,
these will only be used together with peptide IA or IB
compounds. For the sake of clarity, it is emphasised
that compounds of formulae IIA, IIB and IIC are included
in such use.

According to a further feature of the invention we
provide the peptides of formulae IA and/or IB as defined
above optionally in combination with peptides of formula
IC as defined above.

According to a still further aspect of the
invention we provide the use of the compounds of
formulae IA and/or IB as defined above, optionally
together with peptides of formula IC, for the
preparation of pharmaceutical compositions for
prevention or inhibition of binding of tissue factor to
FVII.

As indicated above, the peptides of the sequence of
formula IA are the most active but their activity can be
enhanced synergistically by use together with peptides
of formulae IB and/or IC and fragments and other
derivatives thereof. In such combined use, the peptides
may be simply mixed together or they may be covalently
linked, for example via disulphide bonds between
cysteine residues, or by spacer peptides.

Esters of the peptides of the invention include C_{1-6}
alkyl esters and readily cleaved ester groups such as

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those listed hereinafter as protected carboxyl groups.

Amino acids or peptides attached to the termini of
the peptides of the invention may carry functional
groups such as protecting groups.

Salts of the peptides of the invention include
physiologically acceptable salts such as acid addition
salts such as hydrochlorides.

In the sequences referred to above, the standard
one letter code is used to refer to each naturally
occuring amino acid. This code is standard nomenclature
within the art and can be found in any standard
biochemical textbook such as "Biochemistry" Stryer,
published by W.H. Freeman and Company.

Functional analogues of such peptides are included
within the scope of the present invention. It is well
known within the art that certain amino acids are
functionally equivalent and it has frequently been
observed that exchange of such equivalent peptides
causes no diminution in protein function. Moreover,
substitution of certain non-crucial amino acids may also
be made without any, or any significant, loss of
function - even where those amino acids have been
replaced by chemically dissimilar amino acids. Further,
chemical variants of the naturally occurring amino acids
are known and substitution by such molecules is also
covered by the term "analogue" as used herein.

In the sequence of formula I mentioned above any
"C" representing the amino acid cysteine may be replaced
by alanine (denoted by "A"). Thus, peptides which are
of particular interest include -ENGGA-, -GGAEQ- and
-AEQYV-.

Additionally, peptides derived from the sequence of
formula IA, IB and/or IC include chimeric derivatives
wherein two normally non-adjacent portions (containing
two or more amino acids) of the formula I sequence are
juxtaposed. An example of such a chimeric sequence is
-EQYVNE-.

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Optionally the peptides according to the invention may be cyclic, provided the sequence binding to TF is conformationally available for binding. Cyclisation may be achieved by any suitable chemical means including, for example, formation of disulphide bridges between two cysteine amino acids. One example of a cyclic peptide derived from formula I is CVNENGGCEQYC.

|_____|

The present invention also provides a pharmaceutical composition comprising one or more peptides or analogues or salts thereof, the amino acid sequence of said peptide comprising or being derived from the sequence of formula IA, IB and/or IC above. The peptides may be administered together with any physiologically acceptable excipient known to those skilled in the art, Examples of suitable excipients include water and oil.

The compositions according to the invention may be presented, for example, in a form suitable for oral, nasal, parenteral or rectal administration.

As used herein, the term "pharmaceutical" includes veterinary applications of the invention.

The compounds according to the invention may be presented in the conventional pharmacological forms of administration, such as tablets, coated tablets, nasal sprays, solutions, emulsions, powders, capsules or sustained release forms. Conventional pharmaceutical excipients as well as the usual methods of production may be employed for the preparation of these forms. Tablets may be produced, for example, by mixing the active ingredient or ingredients with known excipients, such as for example with diluents, such as calcium carbonate, calcium phosphate or lactose, disintegrants such as corn starch or alginic acid, binders such as starch or gelatin, lubricants such as magnesium stearate or talcum, and/or agents for obtaining sustained release, such as carboxypolymethylene, carboxymethyl

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cellulose, cellulose acetate phthalate, or
polyvinylacetate.

The tablets may if desired consist of several
layers. Coated tablets may be produced by coating
cores, obtained in a similar manner to the tablets, with
agents commonly used for tablet coatings, for example,
polyvinyl pyrrolidone or shellac, gum arabic, talcum,
titanium dioxide or sugar. In order to obtain sustained
release or to avoid incompatibilities, the core may
consist of several layers too. The tablet-coat may also
consist of several layers in order to obtain sustained
release, in which case the excipients mentioned above
for tablets may be used.

Organ specific carrier systems may also be used.

Injection solutions may, for example, be produced
in the conventional manner, such as by the addition of
preservation agents, such as p-hydroxybenzoates, or
stabilizers, such as EDTA. The solutions are then filled
into injection vials or ampoules.

Nasal sprays may be formulated similarly in aqueous
solution and packed into spray containers either with an
aerosol propellant or provided with means for manual
compression. Capsules containing one or several active
ingredients may be produced, for example, by mixing the
active ingredients with inert carriers, such as lactose
or sorbitol, and filling the mixture into gelatin
capsules.

Suitable suppositories may, for example, be
produced by mixing the active ingredient or active
ingredient combinations with the conventional carriers
envisaged for this purpose, such as natural fats or
polyethyleneglycol or derivatives thereof.

Dosage units containing the compounds of this
invention preferably contain 0.1-10mg, for example 1-5mg
of the peptide of formula (I) or salt thereof.

As indicated above, one aspect of the invention
provides peptides (including the analogues or salts

thereof) according to the invention for use in the
treatment or prevention of blood clotting disorders or
problems. Blood clotting disorders include thrombosis
(particularly vascular thrombosis or deep vein
thrombosis), acute myocardial infarction, restenosis,
reclosure, angina, cerebrovascular disease, peripheral
arterial occlusive disease, hypercoagulability and
pulmonary embolism. The peptides according to the
invention can also be used to prevent occurrence of
blood clotting problems caused by, for example, injury
to blood vessels during thrombolytic therapy, grafting
surgery, vessel patency restoration etc. Blood clotting
disorders may be triggered by sepsis due to production
of TNF-α or IL-1.

In a still further aspect, the present invention
also provides a method of treatment of blood disorders
in the mammalian, preferably human, animal body, said
method comprising administering to said body one or more
peptides of formula IA, IB and/or IC as defined above,
including compounds of formulae IIA, IIB and/or IIC, or
analogues or salts thereof. Prophylactic methods of
treatment are also provided, whereby a peptide according
to the invention is administered to a patient to prevent
or reduce the occurrence of possible blood clotting
problems, for example during surgery or other invasive
techniques. The peptide will of course normally be
administered in the form of a pharmaceutically
acceptable composition.

In another aspect, the present invention provides a
process for the preparation of peptides which comprise
the sequence of formula I or are derived therefrom, or
analogues or salts thereof.

The peptides of the invention may be synthesised in
any convenient way. Generally the reactive groups
present (for example amino, thiol and/or carboxyl) will
be protected during overall synthesis. The final step
in the synthesis will thus be the deprotection of a

protected derivative of the peptide of the invention.

In building up the peptide chains, one can in principle, start either at the C-terminal or the N-terminal although only the C-terminal starting procedure is in common use.

Thus, one can start at the C-terminal by reaction of a suitably protected derivative of, for example lysine with a suitably protected derivative of cysteine or cystine. The lysine derivative will have a free α-amino group while the other reactant will have either a free or activated carboxyl group and a protected amino group. After coupling, the intermediate may be purified, for example by chromatography, and then selectively N-deprotected to permit addition of a further amino acid residue. This procedure is continued until the required amino acid sequence is completed.

Carboxylic acid activating substituents which may, for example, be employed include symmetrical or mixed anhydrides, or activated esters such as for example the p-nitrophenyl ester, 2,4,5-trichlorophenyl ester, N-hydroxybenzotriazole ester (OBt), or N-hydroxy-succinimidyl ester (OSu).

The coupling of free amino and carboxyl groups may, for example, be effected using dicyclohexyl-carbodiimide (DCC). Another coupling agent which may, for example, be employed is N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline.

In general it is convenient to effect the coupling reactions at low temperatures, for example, -20°C up to ambient temperature, conveniently in a suitable solvent system, for example, tetrahydrofuran, dioxan, dimethylformamide, methylene chloride or a mixture of these solvents.

It may be more convenient to carry out the synthesis on a solid phase resin support. Chloro-methylated polystyrene (cross-linked with 1% divinyl benzene) is one useful type of support; in this case

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the synthesis will start at the C-terminal, for example
by coupling N-protected lysine to the support.

A number of suitable solid phase techniques are
described by Eric Atherton, Christopher J. Logan, and
Robert C. Sheppard J. Chem.Soc. Perkin I, 538-46
(1981); James P. Tam, Foe S. Tjoeng, and R. B.
Merrifield J. Am. Chem. Soc. 102 6117-27 (1980); James
P. Tam, Richard D. Dimarchi and R. B. Merrifield Int.
J. Peptide Protein Res 16 412-25 (1980); Manfred Mutter
and Dieter Bellof, Helvetica Chimica Acta 67 2009-16
(1984).

A wide choice of protecting groups for amino- acids
are known and are exemplified in Schröder, E., and
Lübke, K., The Peptides, Vols. 1 and 2, Academic Press,
New York and London, 1965 and 1966; Pettit, G.R.,
Synthetic Peptides, Vols. 1-4, Van Nostrand, Reinhold,
New York 1970, 1971, 1975 and 1976; Houben-Weyl,
Methoden der Organischen Chemie, Synthese von Peptiden,
Band 15, Georg Thieme Verlag, Stuttgart 1974; Amino
Acids, Peptides and Proteins, Vol.4-8, The Chemical
Society, London 1972, 1974, 1975 and 1976; Peptides,
Synthesis-physical data 1-6, Wolfgang Voelter, Eric
Schmidt-Siegman, Georg Thieme Verlage Stuttgart, NY,
1983; The Peptides, Analysis, synthesis, biology 1-7,
Ed: Erhard Gross, Johannes Meienhofer, Academic Press,
NY, San Fransisco, London; Solid phase peptide synthesis
2nd ed., John M. Stewart, Janis D. Young, Pierce
Chemical Company.

Thus, for example amine protecting groups which may
be employed include protecting groups such as
carbobenzoxy (hereinafter also designated Z)
t-butoxycarbonyl (hereinafter also designated Boc),
4-methoxy-2,3,6-trimethylbenzene sulphonyl (Mtr) and
9-fluorenylmethoxycarbonyl (hereinafter also designated
Fmoc). It will be appreciated that when the peptide is
built up from the C-terminal end, an amine-protecting
group will be present on the α-amino group of each new

residue added and will need to be removed selectively
prior to the next coupling step. One particularly
useful group for such temporary amine protection is the
Fmoc group which can be removed selectively by treatment
with piperidine in an organic solvent.

Carboxyl protecting groups which may, for example
be employed include readily cleaved ester groups such as
benzyl (Bzl), p-nitrobenzyl (ONb), pentachlorophenyl
(OPClP), pentafluorophenyl (OPFP) or t-butyl (OtBu)
groups as well as the coupling groups on solid supports,
for example methyl groups linked to polystyrene.

Thiol protecting groups include p-methoxybenzyl
(Mob), trityl (Trt) and acetamidomethyl (Acm).

It will be appreciated that a wide range of other
such groups exists as, for example, detailed in the
above-mentioned literature references, and the use of
all such groups in the hereinbefore described processes
fall within the scope of the present invention.

A wide range of procedures exists for removing
amine- and carboxyl-protecting groups. These must,
however, be consistent with the synthetic strategy
employed. The side chain protecting groups must be
stable to the conditions used to remove the temporary
α-amino protecting group prior to the next coupling
step.

Amine protecting groups such as Boc and carboxyl
protecting groups such as tBu may be removed simul-
taneously by acid treatment, for example with trifluoro
acetic acid. Thiol protecting groups such as Trt may be
removed selectively using an oxidation agent such as
iodine.

The following Examples are given by way of
illustration only.

Figure 1 illustrates the inhibition of blood
clotting by different FVII peptides and FVII peptide
analogues.

Figure 2 shows the dose-response relationship

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between FVII/TF activity and the peptide FVII-5.

Figure 3 is the dose-response relationship between FVII/TF activity and the peptide FVII-5d.

Figure 4 shows the inhibition of Factor X activation at different concentrations of FVII-5.

The present invention is further illustrated by the following, non-limiting, examples.

The following abbreviations are used in the Examples:

TFA   : trifluoroacetic acid

BocAA : t-butoxy carbonyl protected Amino acid

BOP   : benzotriazol-1-yloxy-tris-(dimethylamino)-
              phosphonium-hexafluorophosphate

HOBT   : N-hydroxybenzotriazole

DIEA   : diisopropylethylamine

DCM   : dichloromethane

NEM   : N-ethylmaleimide

EDTA   : ethylenediaminetetraacetic acid

DMF   : dimethylformamide

EXAMPLE 1

General Peptide Synthesis

The peptides used were synthesized by standard procedures of solid phase chemistry and purified by preparative HPLC. The purity of each peptide was checked by analytical HPLC, amino acid analysis and mass spectrometry. All were products of Neosystems Laboratoire (Strasbourg, France).

The peptides were assembled and cleaved using the following general procedure.

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The resin used was 4-methylbenzhydrylamine (batch R2161) with an initial loading of 0.9 meq/g. For each peptide 450 mg of starting resin (0.4 mmol) was used.

1.   Assembling

After neutralisation, the coupling/deprotection protocol described below was used (volume: 10 ml for each solvent).

a) Neat TFA	1 min.	
b) Neat TFA	3 min.	
c) Flow wash methylene chloride		
d) Isopropanol	0.5 min.	
e) Dimethylformamide (DMF)	0.5 min (3 times)	
f) Dimethylformamide flow wash		

Coupling
5 eq. of BocAA, BOP and HOBT were dissolved in 5 ml of DMF and added to the resin. After bubbling started 0.5 ml of diisopropylethylamine was added and agitation was continued for 13 minutes. After 2 DMF washes a double coupling was performed in the same conditions.

Acetylation
After the last deprotection step, acetylation was performed using 10 eq. of acetic anhydride and 10 eq. of DIEA in 10 ml of DMF.

Reaction time: 10 minutes.

Once assembling was achieved, the peptide resin was washed with DMF, DCM ether and then dried under nitrogen stream.

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2.   Cleavage

The peptide was cleaved from the resin and deprotected
by a treatment with HF/anisole (9/1 by volume. 10 ml per
gram of peptide resin) at 0°C for 45 minutes.

After evaporation of the HF and precipitation with
ether, the crude peptide was solubilised in neat TFA and
filtered.

The TFA was then evaporated under reduced pressure and
the peptide was again precipitated with ether. At this
stage the product was ready to be purified.

3.   Purification and analysis

Each peptide was HPLC purified on reversed phase C_{18}
column (15-25μm) using a linear gradient of
acetonitrile/ water (0.1 % TFA). The fractions with a
purity >95% were pooled and lyophilised. The purified
peptide was then analysed by HPLC and a sample was
hydrolized for amino acid analysis. The results were
noted on the analytical data sheet which was sent with
each peptide.

The basic strategy used for purification was always the
same. Only the gradiant used for the preparative
purification was different, depending on the initial
retention time of each individual peptide.

EXAMPLE 2

PEPTIDE FVII-5

The resin used was Boc-Cys(4-MeBzl)-PAM resin (batch
52172) with an initial loading of 0.63 meq/g. 0.96 g of

the starting resin (0.6 mmol) was used.

The assembling/coupling/cleavage procedure used was the same as described above, with the following modifications:

1.   Assembling

    a) TFA 55% in DCM   5 min.
    b) TFA 55% in DCM   25 min.

2.   Cleavage

After precipitation with ether, the peptide resin was washed with 30 ml 10% acetic acid in water and lyophilised.

3.   Purification - cyclisation

Solvent composition :

A : water 0.1% TFA
B : acetonitrile/solvent A (60/40 by volume).

The crude product was pre-purified on reversed phase HPLC using a linear gradiant of acetonitrile/water (0.1% TFA), 10 to 80% of B, 30 minutes.

All the fractions containing the linear peptide with a purity > 80 were pooled and the volume was adjusted to 0.5 litre with water. The pH of the solution was adjusted to 8.5 with DIEA and left overnight at ambient temperature, under magnetic stirring. The evolution of the cyclisation reaction was followed by co-injection of a sample of the reaction mixture with N-ethylmaleimide (NEM).

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After 24 hours, cyclisation was complete. The pH was
then decreased to 2.5 with acetic acid and the solution
concentrated under reduced pressure before
lyophilisation.

The cyclic peptide was then purified up to 95% by RP -
HPLC (linear gradiant from 5 to 30% of B in 30 minutes).

Purity and identity of the final product were then
controlled by analytical HPLC, amino acid analysis and
mass spectrometry.

EXAMPLE 3

PEPTIDE FVII-1

This peptide was synthesised using Fmoc/t-But strategy
starting from 90 mg of Fmoc-Ala-Wang resin (0.67 meq/g).

1.   Assembling

The following protocol was used for deprotection/
coupling :

a)   Piperidine 25% in DMF   2 min.
b)   Flow wash DMF
c)   Piperidine 25% in DMF   4 min.
d)   Flow wash DMF
e)   Piperidine 25% in DMF   6 min.
f)   Flow wash DMF   repeat 5 times.

2.   Coupling

5 eq. of Fmoc-AA, BOP and HOBT were dissolved in 3 ml of
DMF and added to the resin. After bubbling started,

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0.13 ml of DIEA was added and coupling was continued for
13 minutes. After 2 DMF washes, a double coupling was
performed as described above.

3.   Cleavage

Cleavage of the peptide and deprotection were achieved
by a treatment with 5 ml of a mixture of TFA (83.3 %),
water (4.2%), thioanisole (4.2%), ethandithiol (2.1 %)
and phenol (6.25%) for 2.5 hours. After filtration this
mixture was poured into 25 ml of cold ether. The
precipitated peptide was centrifuged and washed twice
with ether. The product was then dissolved in water,
lyophilized and used without further purification.

EXAMPLE 4

Other synthetic peptides were produced by analogy to the
procedure described in Example 1.

Table 1 lists all the peptides synthesised.

TABLE 1 - FACTOR VII PEPTIDES

DESIGNATION	SEQUENCE	RESIDUES IN FVII
FVII-12	LFWISYSD	39-46
FVII-21	WISYSDGD	41-48
FVII-23	SYSDGD	43-48
FVII-2	YSDGDQC	44-50
FVII-7 (cyclic)	CASSPCQNGGSC	50-61
FVII-13	KDQLQSYI	62-69
FVII-8 (cyclic)	CLPAFEGRNC	72-81
FVII-9	NCETHKDDQLICV	80-92

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FVII-5(linear)	CVNENGGCEQYC	91-102
FVII-5(cyclic)	CVNENGGCEQYC	91-102
5A	VNENG	92-96
5B	ENGGA	94-98
5C	GGAEQ	96-100
5D	AEQYV	98-102
5E	EQYVNE	99-101+92-94
FVII-10	NGGCEQYCSD	95-104
FVII-10A	GGCEQYCSD	96-104
FVII-10B	GCEQYCSD	97-104
FVII-10C	CEQYCSD	98-104
FVII-10D	EQYCSD	99-104
FVII-4	SDHTGTKRSCR	103-113
FVII-4B	SDHTGTKRS	103-111
FVII-6(cyclic)	CHEGYSLLADGVSC	114-127
FVII-1	GKIPILEKRNA	136-146
FVII-11	DKIKNWRNLIA	196-206
FVII-3	VGHFGV	371-376

EXAMPLE 5

Assay of FVIIa/TF activity

Peptides (0-1 mM) were preincubated in 0.1 M Tris.HCl pH
7.2 with tissue factor (Thromborel®, Behringwerk,
Marburg, Germany; 0.2% final concentration) at 22°C for
30 minutes, then Factor X (American Diagnostica Inc.,
Greenwich, CT USA; 70 nM was added followed by FVIIa
(Diagnostica Stago, Asnières, France; 8 pM and $CaCl_2$ (5
mM). Incubations were continued for 30 minutes and
quenched with EDTA (50 mM) which removes essential Ca^{2+}.
The FXa produced was quantified by monitoring hydrolysis
of the chromegenic FX substrate S-2222 (Chromogenix,
Mölndal, Sweden).

The results are shown in Figure 1.

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EXAMPLE 6

The effect of different concentrations of the peptide
FVII-5 was investigated using the assay procedure
described in Example 5. The experiment was repeated
using the peptide FVII-5D.

The results are shown in Figures 2 and 3 respectively.

EXAMPLE 7

The kinetics of inhibition of FX activation by FVII-5
was investigated. Varying concentrations of FVII-5 were
incubated with different concentrations of Factor X in
the presence or absence of TF and FVIIa.

The results are shown in Figure 4. Both graphs are
based upon the same data and both indicate a non-
competitive inhibition of Factor X activation by FVII-5
thus indicating that inhibition occurs at an earlier
step than Factor X binding ie. inhibition occurs at
FVIIa/TF complex formation.

EXAMPLE 8

Assay of apoTF/FVIIa Catalytic Activity

This colorimetric assay was used to measure directly the
amidolytic (catalytic) activity of purified human FVIIa
when bound to recombinant human TF in the absence of
phospholipid.

PROTOCOL

Reactions were performed in microtitre plate wells,

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using a final incubation volume of 200 uL. Test
compounds, dissolved in water, (final concentration, 0-1
mM) were preincubated with recombinant human tissue
factor (American Diagnostica, cat# 4500; 5 nM) and $CaCl_2$
(5 mM) in Tris buffer (100 mM), pH 7.2, containing NaCl
(150 mM) and BSA (1 mg/mL) at ambient temperature for 30
minutes. Factor Vlla (Enzyme Research Laboratories,
cat# HFVlla; 5 nM) was added and the incubations
continued for 60 minutes. Chromogenic substrate, S2288
(Chromogenix cat# 820852; 0.5 mM) was then added and the
enzymatic activity of the tissue factor apoprotein/
factor VII complex (apoTF/FVlla) was monitored at 405
nm.

EXAMPLE 9

Assay of HT1080 Cell Surface TF/FVIIa Mediated
Activation of FX

This assay system was used to measure the catalytic
activity of native TF/FVIIa complex on the surface of
living cells expressing TF in their cell membrane. The
activity was measured as the amidolytic (catalytic)
activity of FXa produced by the action of the cell
surface TF/FVIIa on added FX. The cell line chosen for
this assay was the human fibrosarcoma HT-1080 (American
Type Culture Collection CCL 121).

PROTOCOL

HT-1080 cells were suspended in a small volume of serum-
containing medium and mixed with microcarriers (Cytodex-
3, Pharmacia) in a ratio of about 50-100 cells/ micro-
carrier. The cells were usually adherent on the
microcarriers within 2 h and could then be transferred
to normal roller bottles with a larger volume of culture

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medium. The microcarrier cell preparations were then
washed twice in calcium free buffer to remove bound
serum factors. Of nine different washing buffers
tested, 'Hank's buffered salt solution' was found to be
optimal when considering i) low carry-over of serum
factors, ii) small effect on cell adhesion to
microcarrier spheres, iii) little damage to cell
membranes, and iv) high cell surface TF activity. The
microcarrier spheres were resuspended in Hank's buffer
and the beads were counted.

FVII (Enzyme Research Laboratories cat# HVlI 1007; 5 pM)
(and inhibitor in inhibition assays) was then added and
the mixure allowed to equilibrate for 30 minutes before
addition of FX (Enzyme Research Laboratories cat# HFX
1010; 50 nM) and $CaCl_2$ (5 mM). The rate of FX formation
was determined by an amidolytic assay using chromogenic
substrate S2765 for FXa (Chromogenix cat# 821413) and
measurement of the absorbance increase at 405 nm. The
TF activity was easily adjusted by simple dilution of
the microcarrier suspension. The variation could be
kept at a minimum by stirring the suspension when
aliquots were removed. It was determined as $14\pm7\%$.

That FX activation by the cells was truly dependent upon
cell-surface TF could be shown by the use of a
neutralizing monoclonal antibody to human TF as depicted
in Fig 5. Titration of cell-bound TF with FVII
indicated about 11,000 TF molecules per cell or 0.8
$ng/10^6$ cells. Also FX activation was dependent on
incubation time and FVII added (Fig. 6 & 7). That the
activity measured represented FXa was verified by
inhibition of activity with TAP (tick anticoagulant
polypeptide; G. Vlasuk, Corvas Int.) (Fig. 8).

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FIGURE LEGENDS

Fig. 5 Inhibition of the cell mediated activation of
       factor X by a neutralizing monoclonal antibody to
       tissue factor (American Diagnostica #4504). HT-
       1080 cells and antibody were preincubated for 1
       hour at room temperature before the addition of
       factor VII (5 pM) and factor X (56 nM).
       Reactions were continued for 2 hours and quenched
       by the addition of EDTA. Formation of factor Xa
       was monitored by an amidolytic assay using a
       factor Xa chromogenic substrate (Chromogenix,
       S2765). Inhibition reached >95% indicating
       complete dependence on tissue factor.

Fig. 6 Time course for factor X activation using 0 (■)
       and 1 nM (●) factor VII.

Fig. 7 Dependence of cell mediated factor X activation
       on added factor VII. Mean of triplicate
       determinations. Intra-assay variation was 12±14%.

Fig. 8 Inhibition by TAP (tick anticoagulant
       polypeptide) of FXa amidolytic activity generated
       by HT-1080 cell-surface TF/FVIIa.

EXAMPLE 10

Assay of Cell-surface TF Initiated Coagulation of Plasma

A novel assay system has been devised to measure the
coagulation of blood plasma (i.e. clotting time) induced
by tissue factor (TF) expressed on the surface of living
cells. This assay makes use of human or animal cells
adherent on the surface of microcarriers (see Fig 9),
which enables reproducible presentation of known amounts

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of TF activity in the stirred measuring well of an
electromagnetic coagulometer. The cells used may be
normal or transformed, and representing phenotypes such
as monocytes, endothelium, fibroblasts etc (see Fig 10).

This novel assay has been particularly developed and
characterised so as to be highly useful in determining
the effect of synthetic peptides and peptidomimetic
compounds on the extrinsic pathway of coagulation, using
living cells in a plasma milieu. Results for the
inhibitory effect of synthetic peptides based upon the
amino acid sequence of human FVII are disclosed.

PROTOCOL

The microcarrier cell preparation is made as follows.
Cells obtained from primary isolates or subcultures of
transformed lines (see Fig. 10) are suspended in a small
volume of serum-containing medium and mixed with
microcarriers (Cytodex-3, Pharmacia) in a ratio of about
50-100 cells/microcarrier. The cells are usually
adherent on the microcarriers within 2 hours, and can
then be transferred to normal roller bottles with a
larger volume of culture medium. Uniform coverage of
microcarriers with about 20-60 cells can be obtained
directly with transformed cells, when large cell numbers
are available, or after 2 days growth when primary
cultures (e.g. umbilical vein endothelial cells) are
used. Primary cultures of cells not expressing TF (e.g.
monocytes or endothelial cells) may be stimulated to
express TF by incubation of the adherent cells with such
agents as bacterial lipopolysaccharide (LPS) or tumour
necrosis factor-alpha (TNF-α) (see Fig. 10).

To assay the extrinsic coagulation activity, the
microcarrier cell preparations are first washed with a
calcium-free buffer to remove bound serum factors. A

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calcium-containing buffer and plasma are then added and
the clotting time is determined automatically in an
electromagnetic coagulometer. The clotting time may be
very conveniently adjusted (e.g. 35 sec) by simple
dilution of the microcarrier suspension, and
reproducibility is easily obtained provided the
suspension is stirred when aliquots are removed. The
clotting time may be converted to arbitrary TF units by
a log-log calibration curve generated with rabbit brain
thromboplastin.

The system obtained can be shown to faithfully represent
the extrinsic pathway of coagulation, by the use of a
panel of immunodepleted human plasmas deficient in each
of the coagulation factors. Coagulation mediated by
e.g. ECV304 human endothelial cells (American Culture
Collection CRL-1998) is clearly dependent on FVII and FX
(Fig. 11). Thus there is no direct activation of FX;
the coagulation is mediated by cell-surface TF/FVI1.
That this procoagulant activity really represents TF-
mediated coagulation was demonstrated by the use of a
neutralising monoclonal antibody to human TF, which
could abolish all coagulation activity (Fig 12.

FIGURE LEGENDS

Fig. 9   Principle of the microcarrier assay of cell-
         surface mediated coagulation of blood plasma.
         Living cells (e.g. human HT-1080 fibrosarcoma;
         ATCC CRL 121) adherent on the surface of
         microcarriers are mixed with a calcium-
         containing buffer and plasma in the incubation
         well of a Thrombotrack™ electromagnetic
         coagulometer (Nycomed). The clotting time is
         measured by the increase in resistance to
         movement of a magnetic ball produced by the
         formation of fibrin fibres. HT-1080 cells

adherent on Cytodex-3 microcarriers are shown in
the lower inset as an example of the types of
cells expressing TF which may be used.

Fig. 10 Coagulant activity of a selection of cell types
adherent on microcarriers. The activity is
expressed as TF units per 10^5 cells, calculated
from a calibration curve generated with rabbit
brain thromboplastin (Nycomed). The cell types
shown are: RD, human rhabdomyosarcoma cells
(ATCC CCL 136); HT-1080, human fibrosarcoma
cells (ATCC CRL 121); HE US, unstimulated
primary culture of human umbilical vein
endothelial cells; HE ST, human umbilical vein
endothelial cells after stimulation with
bacterial lipopolysaccharide (1 μg/ml; 5 h);
ECV-304, human endothelial cells (ATCC CRL
1998); HM US, unstimulated human peripheral
monocytes and HM ST, human peripheral monocytes
after stimulation with bacterial
lipopolysaccharide (0.5 μg/ml; 2 h).

Fig. 11 Dependence of ECV-304 mediated coagulation on
specific coagulation factors, demonstrated by
the use of immunodepleted human plasmas
(American Diagnostica) in the microcarrier
assay. Note complete dependence on factors VII
and X.

Fig. 12 Characterisation of the coagulant activity on
ECV-304 cells in the microcarrier assay by the
use of a neutralising monoclonal antibody to
human TF (American Diagnostica cat#4504).
Almost all clotting activity can be abolished by
the neutralising antibody, thus demonstrating
that it is highly dependent on the function of
TF.

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EXAMPLE 11

Inhibition of TF/FVII Assay by FVII Peptides

ELISA Assay

The inhibition results shown in Fig. 13 indicate that in
addition to sequences from the "hinge" region (i.e.
residues 41-48 between the GLA domain and the first EGF
domain, UNIVERSITY OF TEXAS) and from the first EGF
domain itself (i.e. residues 50-61; Clarke et al), there
exist additional sequences in the second EGF domain
(especially 95-104) which are inhibitors when presented
as peptides in this binding assay. Peptide 95-104 was
considerably better as an inhibitor in this assay than
103-111 (CORVAS Int.)

Colorimetric Assay of apoTF/FVIIa Amidolytic Activity

Tests using this type of assay further demonstrated that
the peptide representing residues 95-104 of human FVII
is a good inhibitor of TF/FVII complex formation, and
better than the peptide 103-111 (CORVAS Int.) (see Figs.
14 and 15). Note that residue 95 was an important
contributor to the inhibitory properties of the peptide
95-104, shown by the very low activity of peptide 96-104
compared to peptide 95-104 (Fig.14). Synergism was also
apparent when certain peptide mixtures were tested as
inhibitors, e.g. a mixture of 95-104 and 72-81 (see Fig.
16). Kinetic experiments demonstrated that inhibition
by peptide 95-104 was non-competitive with respect to
the catalytic site of FVIIa (see Fig. 17), consistent
with inhibition of TF/FVII complex formation rather than
direct inhibition of FVIIa's enzyme activity.

Activation of FX by Cell-Surface TF/FVIIa

Peptide 95-104 was clearly the best inhibitory peptide
of those tested in this assay also, and considerably
better than 103-111 (CORVAS Int.) (Fig. 18). Note that
residues 95-97 were important contributors to the
inhibitory properties of the peptide 95-104.

Coagulation Initiated by Cell-Surface TF/FVIIa

This assay system differed critically from those above
in that the reaction mixture contained whole human
plasma, and therefore represented considerably more
closely the environment of the luminal surface of an
endothelial cell in vivo. In this system also, peptide
95-104 was a good inhibitor, followed by 41-48 (TEXAS
UNIVERSITY) and 91-102 (Fig. 19). The inhibitory effect
of 95-104 was also clearly dependent on residue 95.
Note that 114-127 was a moderate inhibitor. Once again,
95-104 was a considerably better inhibitor than 103-111
(CORVAS Int.) in this system also (Fig. 20).

FIGURE LEGENDS

Fig. 13 ELISA assay of the inhibition of FVII binding to
immobilized rTF produced by synthetic FVII
peptides (all at 0.5 mM). For peptide sequences
see the table of peptides above (page 19). C
and L denote the cyclic and linear forms
respectively of peptides with terminal
cysteines.

Fig. 14 Colorimetric assay of the inhibition of
apoTF/FVIIa amidolytic activity produced by
synthetic FVII peptides (all at 0.5 mM). C and
L denote the cyclic and linear forms
respectively of peptides with terminal

cysteines.

Fig. 15 Inhibition of apoTF/FVIIa amidolytic activity by
FVII peptide sequences selected by Bioreg (95-
104) and Corvas Int (103-111).

Fig. 16 Synergistic inhibition of apoTF/FVIIa amidolytic
activity. The figure shows inhibition with 95-
104 alone (■), with a mixture of 95-104 and a
fixed concentration (0.5 mM) of 72-81 (▲) and
with 0.5 mM 72-81 alone (●). The theoretical
(additive) effect of an equimolar mixture of the
two peptides is indicated by a solid line
without symbols.

Fig. 17 Kinetic experiment analysing mode of inhibition
of apoTF/FVII activity by peptide 95-104. This
inhibition is clearly non-competitive, thus
demonstrating that the inhibition observed is
not mediated by an effect on the active site of
FVIIa.

Fig. 18 FVII peptide inhibition of activation of FX by
TF/FVlIa on the surface of HT-1080 cells.
Results are shown for each peptide at 0.5 mM.

Fig. 19 The effect of a selection of synthetic FVII
peptides (all at 0.5 mM) on the coagulation of
plasma by TF/FVIIa on the surface of HT-1080 and
ECV304 cells. The peptide numbers shown refer
to the amino acid sequence of human FVII; C and
L denote the cyclic and linear forms
respectively of peptides with terminal
cysteines.

Fig. 20 Inhibition of ECV-304 cell-surface TF mediated
coagulation by synthetic FVII peptide sequences

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selected by Bioreg (95-104) and Corvas Int (103-111).

The results of the above peptide inhibition experiments are tabulated below:

Summary of Peptide Inhibition Results

FVII peptide IC_{50} (mM) or % inhibition at 0.5 mM[a]
Sequence[b]

	Amidolytic apo TF Assay	Amidolytic Microcarrier assay	ELISA binding assay	Coagulation microcarrier assay
72-81(C)	2.40	0.35	89%	(22 %)
91-102(C)	0.60		0.04	0.58
91-102(L)	1.14		0.06	(14 %)
91-102(anal)[c]	1.30		0.05	1.50
95-104	0.38	0.13	0.03	0.40
96-104	0.92		0.08	(27 %)
98-102				(17 %)
103-111	2.20	0.87	59%	(30 %)
114-127(C)	(21 %)		(77 %)	(44 %)

[a]   Percent inhibition at 0.5 mM is given for those peptides which did not reach quantitative or near-quantitative inhibition at the highest test concentration (1 mM).

[b]   The sequence numbers given are with reference to the native sequence of human FVII. C and L refer to the disulfide cyclised and linear forms respectively.

[c]   91-102(anal) has an arginine residue at position 100, in place of glutamine.

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EXAMPLE 12

Validation of Specificity of Peptide Inhibitors

The effect of the peptides observed in the coagulation
assay could conceivably have been due to effects on
another one or more of the several components
contributing to the coagulation cascade. This
possibility was therefore tested by adding the peptides
to coagulation assays which were initiated by contact
activation (Cephotest™, Nycomed), and therefore involved
coagulation factors of the intrinsic and common pathways
of coagulation, i.e. all coagulation factors other than
TF/FVII. The peptides 95-104, 91-102 and 103-111 were
inactive in this system, while control inhibitors of FX
(TAP) and thrombin (Hirudin; HIR) were strongly
inhibitory (see Fig. 21 below). Thus evidence was
obtained that the effects observed in the TF/FVII
initiated coagulation assay above were in fact due to a
direct effect on the TF/FVII complex.

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Claims

1.   Compounds comprising the amino acid sequences of formulae

5   -CVNENGGCEQYCSD-   IA
            -FCLPAFEGRNCE- and/or   IB
            -RCHEGYSLLADGVSCT-   IC

as well as peptide fragments thereof, esters, amides,
10   salts and cyclic derivatives thereof, functional
analogues thereof and extended peptide chains carrying
amino acids or peptides at the termini of the above
sequences or fragments, for use in the prevention or
inhibition of binding of tissue factor to FVII.

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2.   Compounds comprising the amino acid sequences of
formulae
            -CVNENGGCEQYCSD-   IA
            -FCLPAFEGRNCE- and/or   IB
20   -RCHEGYSLLADGVSCT-   IC

as well as peptide fragments thereof, esters, amides,
salts and cyclic derivatives thereof, functional
analogues thereof and extended peptide chains carrying
25   amino acids or peptides at the termini of the above
sequences or fragments with the exclusion of the
peptides

            CVNENGGCEQYC   IIA
30   CLPAFEGRNC   and   IIB
            CHEGYSLLADGVSC   IIC.

3.   Compounds as claimed in claim 1 or claim 2
comprising 5 or more amino acids.

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4.   Compounds as claimed in any one of claims 1 to 3
being -VNENG-, -ENGGC-, -GGCEQ-, -CEQYV-, -NGGCEQYCSD-
or -GGCEQYCSD-.

5.   Compounds as claimed in any one of claims 1 to 3
being -ENGGA-, -GGAEQ- or -AEQYV-.

6.   A compound as claimed in any one of claims 1 to 3
being -EQYVNE-.

7.   Compounds as claimed in any one of claims 1 to 3
being cyclic derivatives thereof.

8.   Use of compounds of formulae IA and/or IB as
defined in claim 1, optionally together with compounds
of formula IC, for the preparation of pharmaceutical
compositions for prevention or inhibition of binding of
FVII to tissue factor.

9.   Use of compounds of formulae IA and/or IB,
optionally together with compounds of formula IC, for
the prevention or inhibition of binding of FVII to
tissue factor.

10.   Use as claimed in claim 8 or claim 9, wherein the
compounds of formula IA, IB and IC comprise 5 or more
amino acids.

11.   Pharmaceutical compositions comprising one or more
peptides or analogues or salts thereof, the amino acid
sequence of said peptide comprising or being derived
from the sequence of formula IA, IB and/or IC as defined
in claim 1 together with a physiologically acceptable
excipient.

12.   A method of treatment of the human or animal body
to combat or prevent blood disorders said method

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comprising administering to said body one or more
peptides of formula IA, IB and/or IC as defined in claim
1 or analogues or salts thereof.

13.   A process for the preparation of compounds of
formula IA, IB and/or IC as defined in claim 1, said
process comprising the step of deprotecting a protected
derivative of a compound of formula IA, IB and/or IC.

Figure 1   1/21

Inhibitory effect of synthetic factor VII peptide analogs on
factor VII/ tissue factor activity

Factor VII peptides (0.5 mM)

Factor VII peptide analogs (0.8 mM)
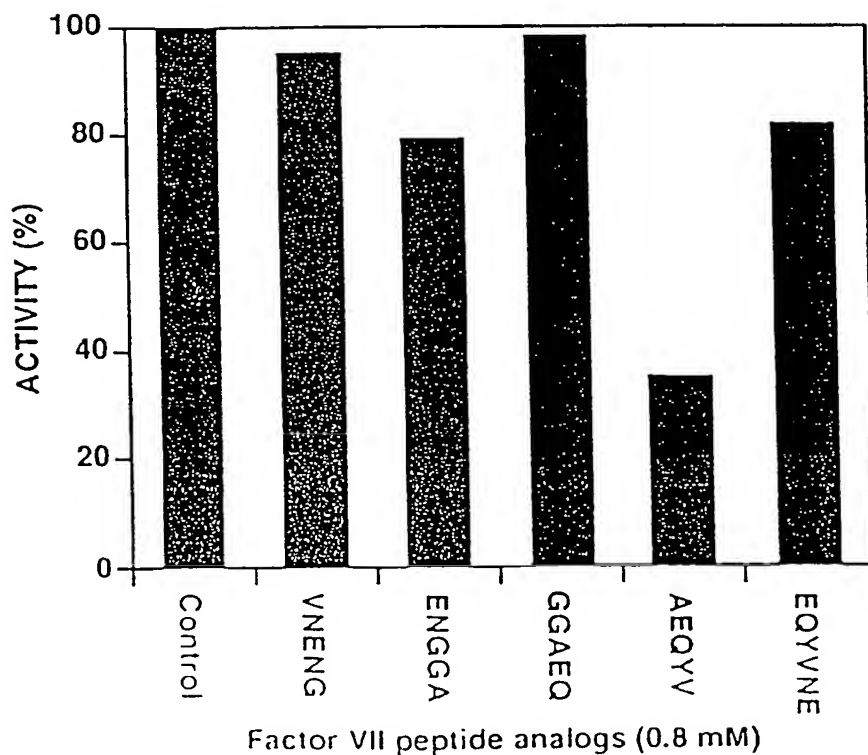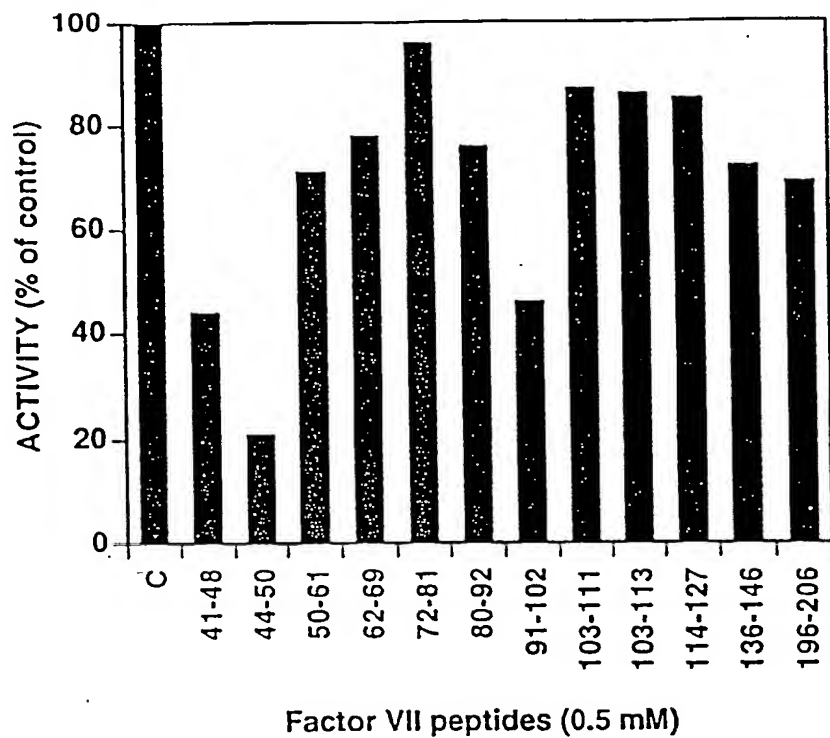
Figure 2
Inhibition of factor VII/TF activity by cyclic peptide VII-5
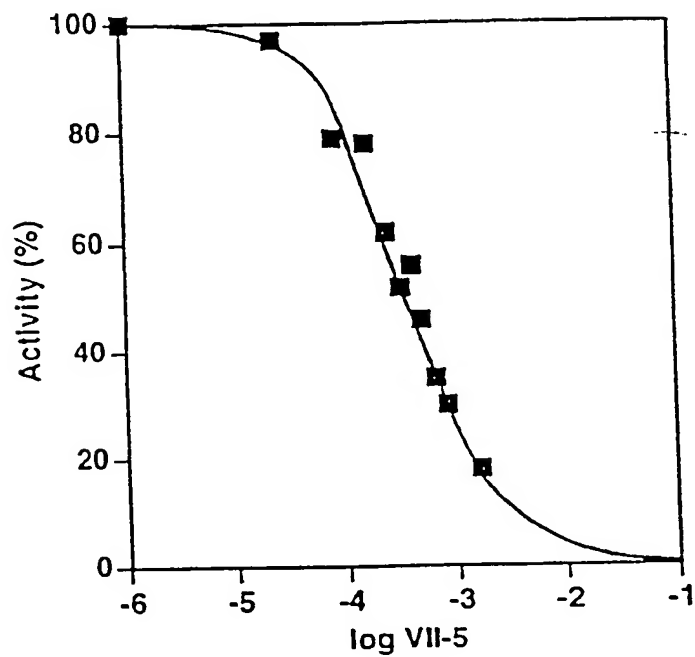
Figure 3

Inhibition of factor VII/TF activity by VII-5D
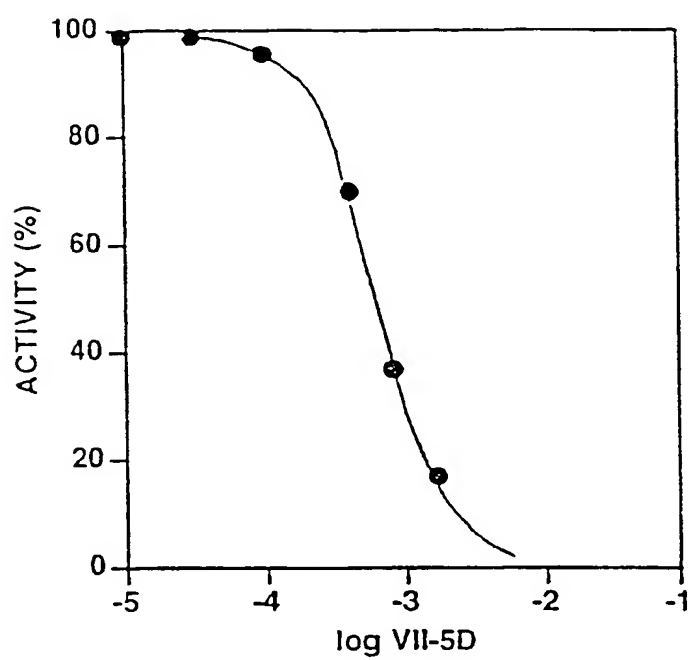
Figure 4

Inhibition of FX activation by VII-5 as a function of FX concentration
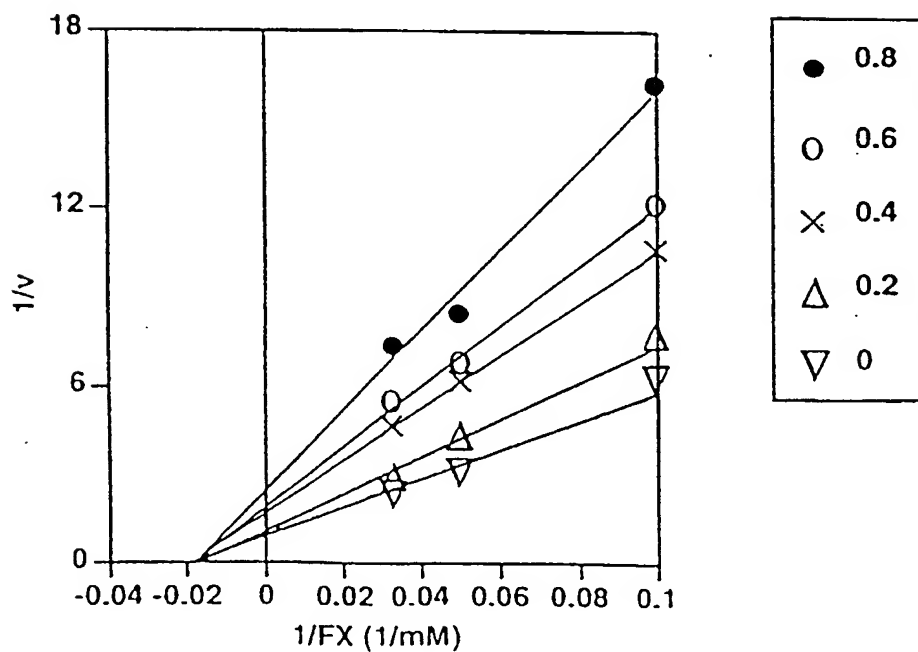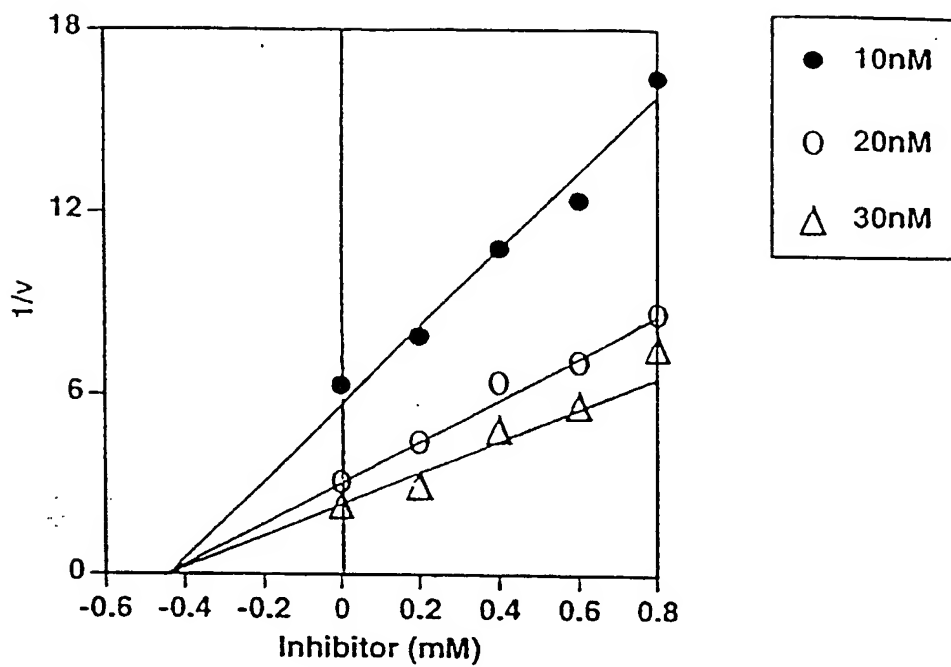
FIGURE 5

INHIBITION OF HT-1080 MEDIATED FXa
FORMATION BY ANTI-TF MAb#4504

FIGURE 6

TIME COURSE OF FX ACTIVATION
BY HT-1080 CELLS

FIGURE 7

DEPENDENCE OF FXa FORMATION
UPON FVII CONCENTRATION
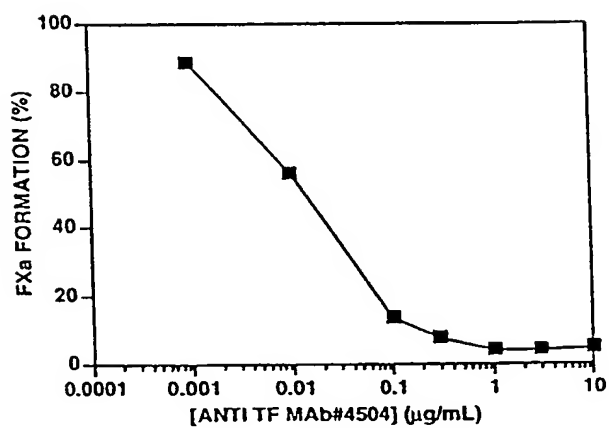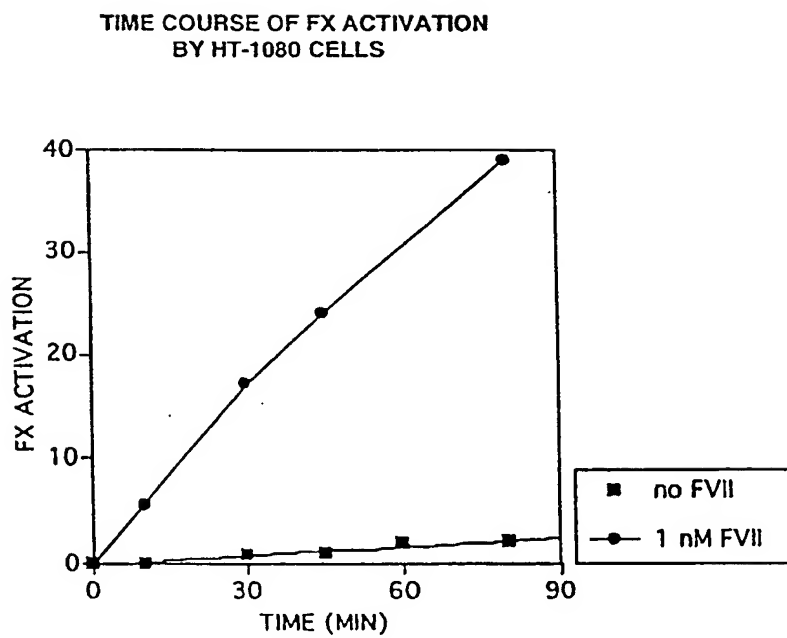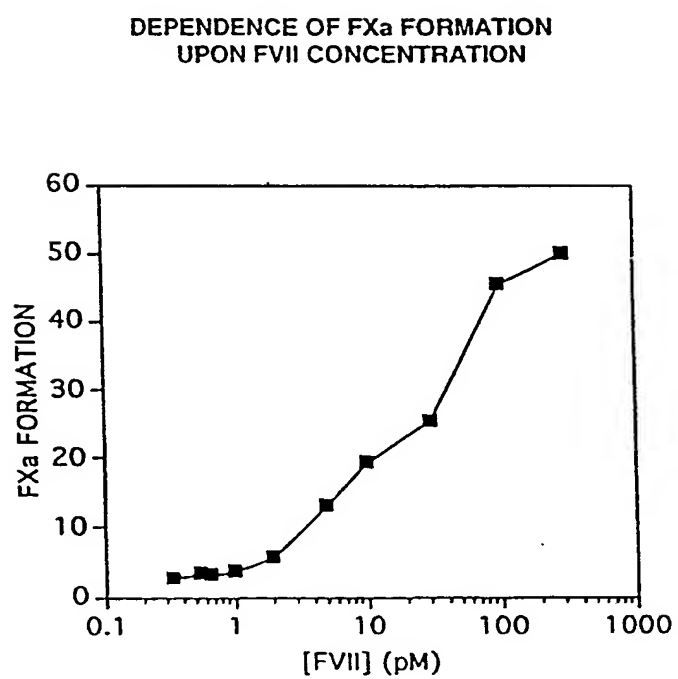
FIGURE 8

TAP INHIBITION OF FXa PRODUCED BY TF/FVIIa
ON HT-1080 CELL-SURFACE
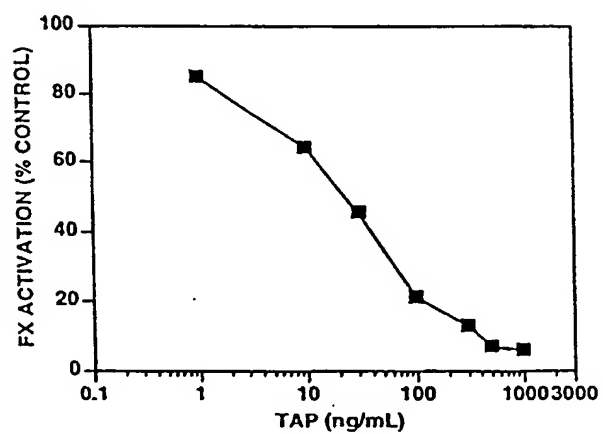
FIGURE 9

MICROCARRIER ASSAY OF CELL SURFACE TF
MEDIATED COAGULATION OF BLOOD PLASMA

TIME o

TIME t
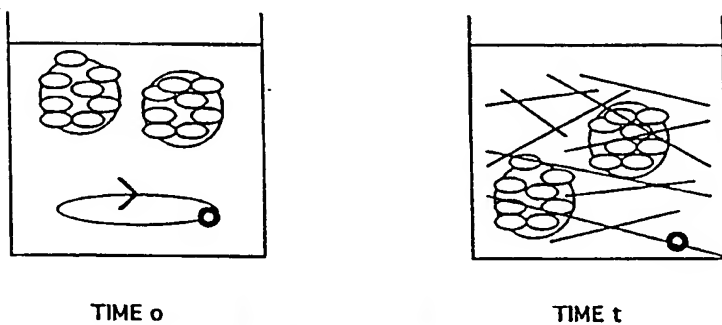
HT-1080 CELLS ADHERENT ON
A CYTODEX-3 MICROCARRIER

FIGURE 10

CELL SURFACE TF MEDIATED COAGULATION
ACTIVITY OF A SELECTION OF CELLS
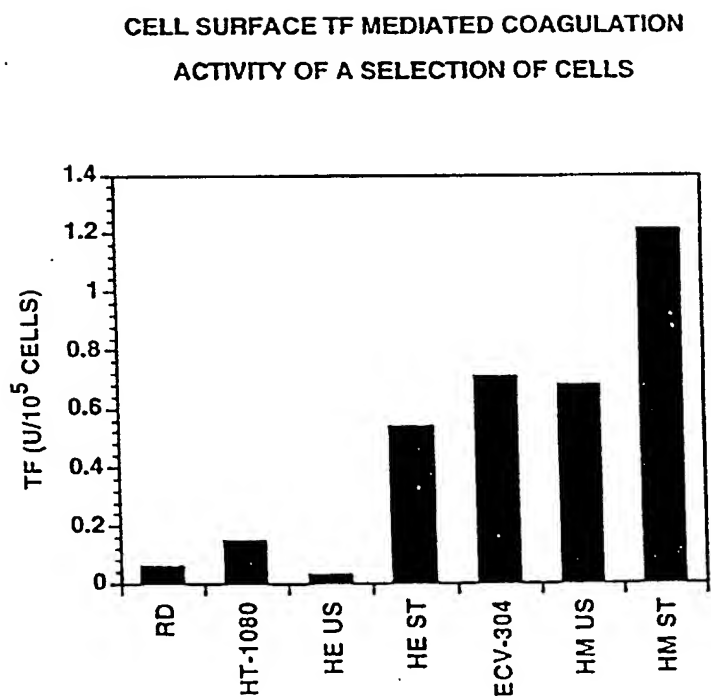
FIGURE II

EFFECT OF SPECIFIC DEPLETION OF COAGULATION
FACTORS ON ECV-304 TF MEDIATED COAGULATION
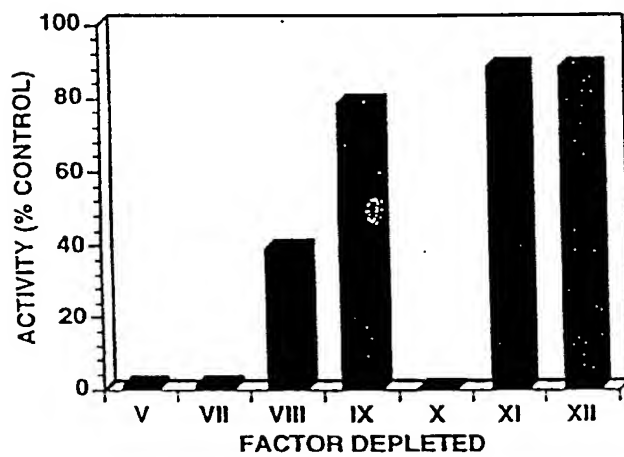
FIGURE 12

INHIBITION OF ECV-304 MEDIATED COAGULATION BY
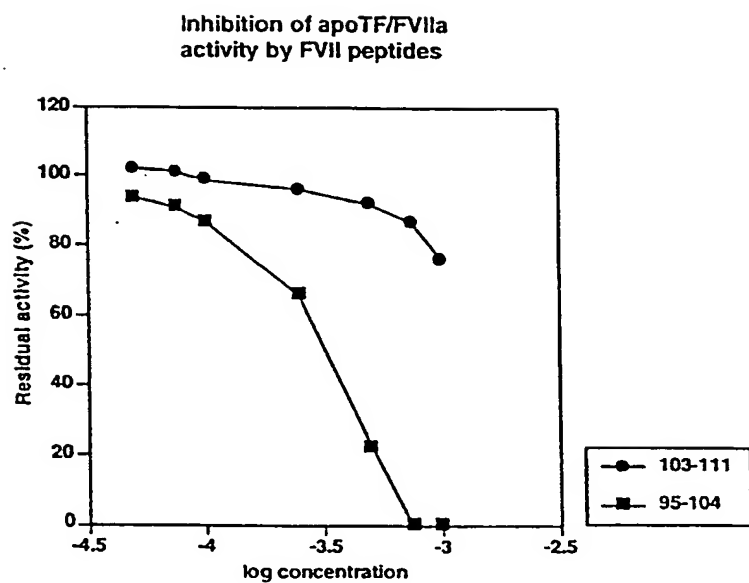MONOCLONAL ANTI-TF ANTIBODY AD 4504

FIGURE 13

INHIBITION OF FVII BINDING TO rTF BY
SYNTHETIC PEPTIDES FROM
THE AMINO ACID SEQUENCE OF HUMAN FVII
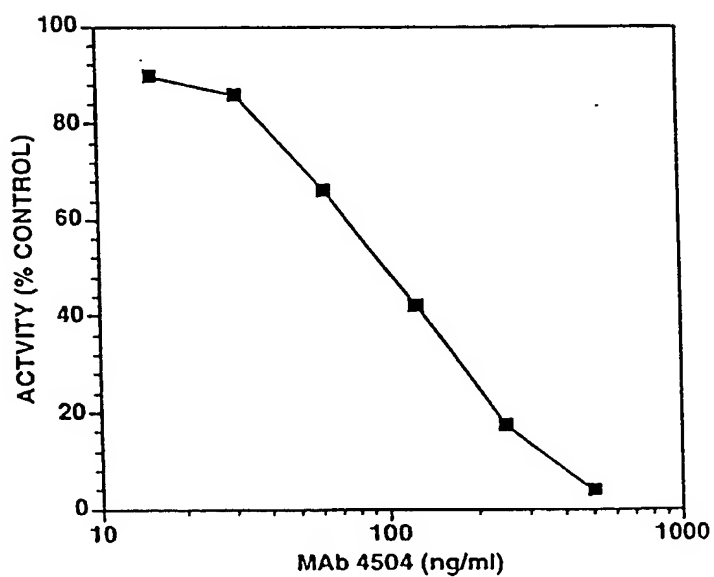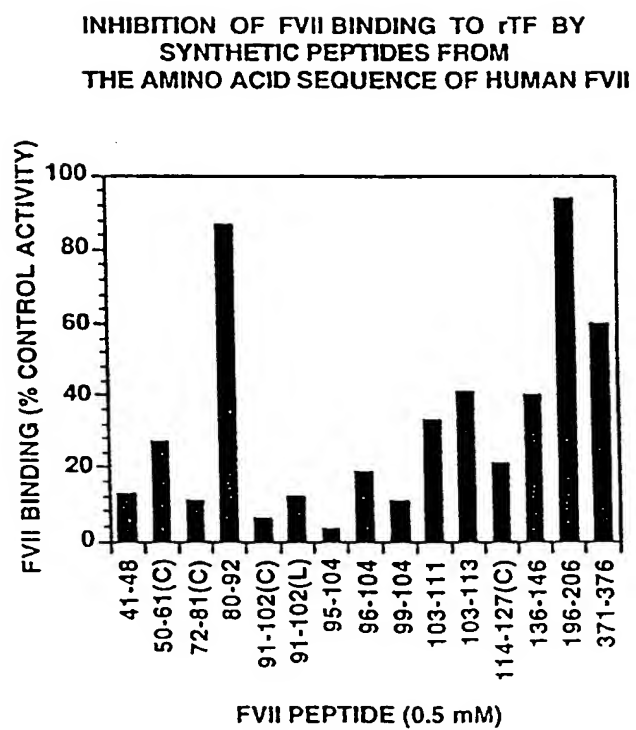
FIGURE 14

INHIBITION OF apoTF/FVIIa ACTIVITY BY
SYNTHETIC FVII PEPTIDES

FIGURE 15

Inhibition of apoTF/FVIIa
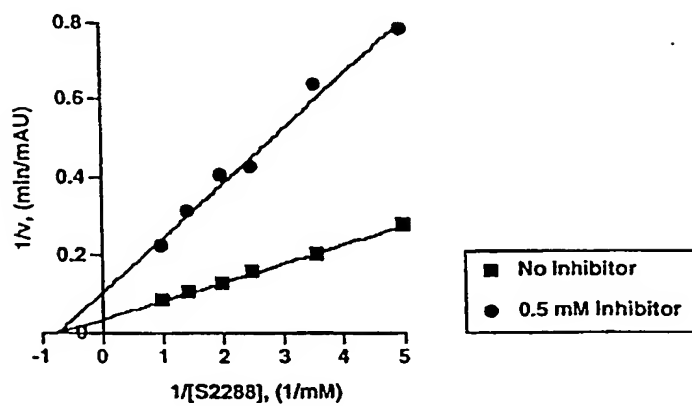activity by FVII peptides
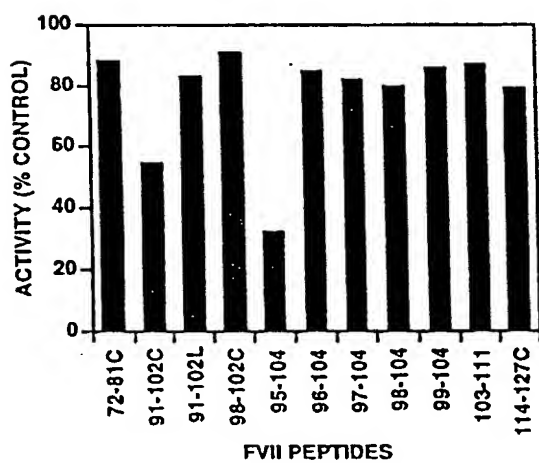
FIGURE 16

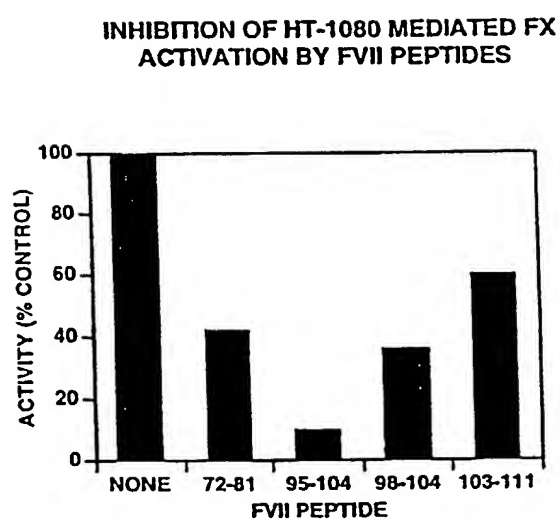**Inhibition of apoTF/VIIa activity:**
Synergy between 95-104 and 72-81(C)
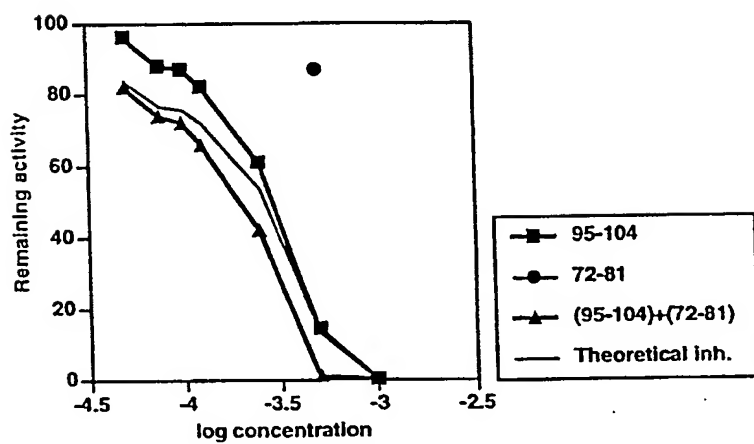
FIGURE 17

EFFECT OF PEPTIDE 95-104 ON APO-TF/FVIIa
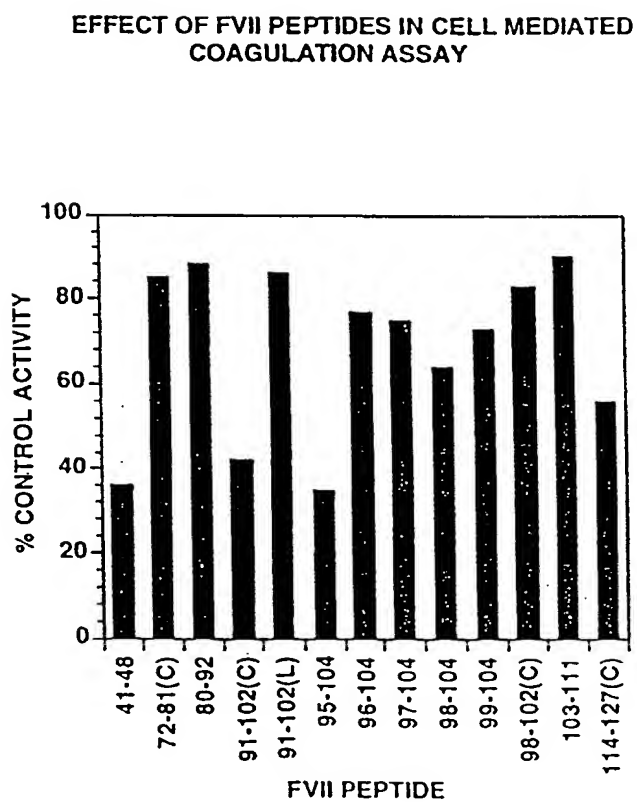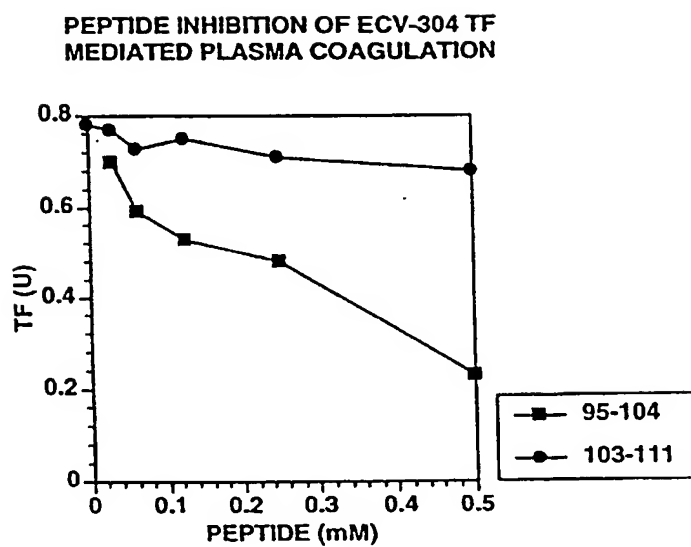DEPENDENT HYDROLYSIS OF S2288
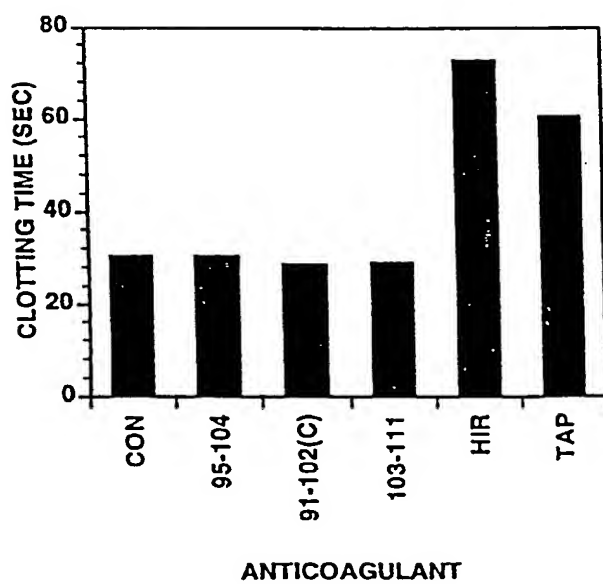
FIGURE 18

**INHIBITION OF HT-1080 MEDIATED FX
ACTIVATION BY FVII PEPTIDES**

FIGURE 19

EFFECT OF FVII PEPTIDES IN CELL MEDIATED
COAGULATION ASSAY

FIGURE 20

**PEPTIDE INHIBITION OF ECV-304 TF
MEDIATED PLASMA COAGULATION**

Figure 21

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K7/06   C07K7/08   A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 09804 (THE SCRIPPS RESEARCH INSTITUTE) 27 May 1993 see sequence no. 9 ---	1-4,9-13
X	EP,A,0 446 797 (BEHRINGWERKE) 18 September 1991 see claim 3 ---	1-13
A	WO,A,90 03390 (CORVAS, INC.) 5 April 1990 cited in the application see the whole document -----	1-13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 1994	2 1. 10. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claims 9-10, 12 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9309804	27-05-93	NONE		
EP-A-0446797	18-09-91	DE-A-	4007902	19-09-91
		AU-A-	7275891	19-09-91
		JP-A-	4217996	07-08-92
		US-A-	5254672	19-10-93
WO-A-9003390	05-04-90	AU-A-	4338689	18-04-90
		EP-A-	0391999	17-10-90